CLAIMS

1. A method of isolating at least one anti-ligand to at least one target ligand comprising the steps of:

(i) providing a library of anti-ligands;

(ii) providing an amount of a first subtractor ligand;

(iii) providing an amount of a second target ligand;

(iv) determining amounts of first subtractor and second target ligands using one or more equations derived from the universal law of mass action $\frac{[C]^c[d]^d}{[A]^a[B]^b} = K_{eq}$,

where:

A, B, C & D = are the participants in the reaction (reactants and products)

a, b, c, & d = the coefficients necessary for a balanced chemical equation

so as to permit isolation of at least one anti-ligand to at least one target ligand;

(v) providing the amount of the first subtractor ligand determined in step (iv);

(vi) providing the amount of the second target ligand determined in step (iv);

(vii) providing separation means for isolating anti-ligand bound to the second target ligand from anti-ligand bound to the first subtractor ligand;

(viii) exposing the library of (i) to the ligands of (v) and (vi) to permit binding of anti-ligands to ligands; and

(ix) using the separation means to isolate the anti-ligand bound to second target ligand.

2. A method of isolating at least one anti-ligand to at least one target ligand comprising the steps of:

(i) providing data set(s) describing a library of anti-ligands;

(ii) providing data set(s) describing a first subtractor ligand;

(iii) providing data set(s) describing a second target ligand;

(iv) automatically determining amounts of the first subtractor and second target ligands using one or more equations derived from the universal law of mass action $\frac{[C]^c[d]^d}{[A]^a[B]^b} = K_{eq}$,

where:

A, B, C & D = are the participants in the reaction (reactants and products)

a, b, c, & d = the coefficients necessary for a balanced chemical equation

so as to permit isolation of at least one anti-ligand to at least one target ligand

(v) providing the amount of the first subtractor ligand determined in step (iv);

(vi) providing the amount of the second target ligand determined in step (iv);

(vii) providing th elibrary of anti-ligands described by the data-set of step (i);

(viii) providing separation means for isolating anti-ligand bound to the second target ligand from anti-ligand bound to the first subtractor ligand;

(ix) exposing the library of (vii) to the ligands of (v) and (vi) to permit binding of anti-ligands to ligands; and

(x) using the separation means to isolate the anti-ligand bound to second target ligand.

3. A method as claimed in Claim 2 wherein step (iv) and at least one of steps (v), (vi), (vii) (ix) and (x) are performed automatically.

4. A method as claimed in one of Claims 1, 2 or 3 comprising a further step of releasing the anti-ligand from the second target ligand.

5. A method as claimed in any previous claim whereby steps (ii) to (ix) are repeated one or more times.

6. A method as claimed in any previous claim wherein the amount of one of the first subtractor or second target ligand is provided in excess of the amount of the other of the first subtractor or second target ligand.

7. A method as claimed in Claim 6 where the excess of ligand is between 10 and 100 fold.

8. A method as claimed in any one of Claims 1 to 7 wherein the equation of (iv) is

$$bA = \frac{(A+T+(K_d)x(CxV))}{2} - \sqrt{\frac{(A+T+(K_d)x(CxV))^2}{4} - AxT}$$

where

bA = Bound anti-ligand

A = Total number of anti-ligand

T = Total number of ligands

C = Avogadro's constant (6.022×10^{23} particles/mole)

V = Reaction volume (litres)

K_d = Equilibrium dissociation constant

9. A method as claimed in any one of claims 1 to 7 wherein the equation of (iv) is:

$$bA = \left\{\frac{(A+T+(K_d)x(CxV))}{2} - \sqrt{\frac{(A+T+(K_d)x(CxV))^2}{4} - AxT}\right\} x \left\{\frac{(T_p x C_p)}{((T_p x C_p)+(T_x C_x))}\right\}$$

where

bA_p = Bound anti-ligand

T_p = The number of ligands on C_p

T_s = The number of ligands on C_s

C_p = The number of target ligand constructs

C_s = The number of subtractor ligand constructs

A = Total number of anti-ligand

T = Total number of ligands

C = Avogadro's constant (6.022×10^{23} particles/mole)

V = Reaction volume (litres)

K_d = Equilibrium dissociation constant

10. A method as claimed in any previous claim wherein the separation means are selected from at least one of a solid support, cell membrane and/or portions thereof, synthetic membrane, beads, chemical tags and free ligand.

11. A method as claimed in Claim 10 whereby the separation means are cell membranes and/or portions thereof.

12. A method as claimed in Claim 11 whereby the first subtractor and second target ligands are fixed to and/or incorporated within separate cell membranes and/or portions thereof.

13. A method as claimed in any previous claim whereby the separation means of the first subtractor and second target ligands have a different density.

14. A method as claimed in Claim 13 wherein the separation means of the first subtractor ligand is of a lower density than the separation means of the second target ligand.

15. A method as claimed in Claim 14 wherein the separation means of the first subtractor ligand is a membrane vesicle.

16. A method as claimed in Claim 14 wherein the separation means of the second target ligand is a whole cell membrane.

17. A method as claimed in any previous claim whereby the isolation of anti-ligand bound to second target ligand is performed by at least one of density centrifugation, solid support sequestration, magnetic bead sequestration, chemical tag binding and aqueous phase partitioning.

18. A method as claimed in Claim 17 whereby the isolation step is performed by density centrifugation.

19. A method as claimed in Claim 18 wherein the density centrifugation is performed using a sucrose-polymer gradient.

20. A method as claimed in any previous claim wherein the library of step (i) is a display library comprising a plurality of library members which display anti-ligands.

21. A method as claimed in Claim 20 wherein the library is a phage display library.

22. A method as claimed in any previous claim wherein the subtractor and target ligands are independently at least one from antigens; receptor ligands; and enzyme targets that comprise at least one selected from carbohydrate; protein; peptide; lipid; polynucleotide; inorganic molecules and conjugated molecules.

23. A method as claimed in any previous claim wherein the library of anti-ligands is composed of at least one selected from antibodies, and antigen binding variants, derivatives or fragments thereof; scaffold molecules with engineered variable surfaces; receptors; and enzymes.

24. A method as claimed in any previous claim comprising a further step of exposing the ligand and its separation means to a stimulus which influences the expression of target ligands on said ligand constructs.

25. A method of screening a library of anti-ligands substantially as described herein preferably with reference to one or more of the accompanying examples and/or figures.

26. A method for preparing a pharmaceutical composition which comprises, following the identification of an anti-ligand with desired characteristics by a method according to any preceding claim, adding said anti-ligand to a pharmaceutically acceptable carrier.

27. A pharmaceutical composition as prepared by the method of Claim 26 for use in medicine.

28. Use of a pharmaceutical composition as claimed in Claim 26 or 27 in the prevention, treatment, imaging, diagnosis or prognosis of disease.

29. A computer program product, comprising a computer readable medium having thereon computer program code means adapted, when said program is loaded onto a computer, to make the computer execute the method of any one of claims 2 to 24.

30. A computer program, distributable by electronic data transmission, comprising computer program code means adapted, when said program is loaded onto a computer, to make the computer execute the method of any one of claims 2 to 24.